

SEAP.GLP-1Gly8

```

      10      20      30      40      50
GAATTCGCCCCACCATGCTGCTGCTGCTGCTGCTGCTGGGCCTGCGCCTG
CTTAAGGCGGGTGGTACGACGACGACGACGACGACGACCCGGACGCGGAC
      M L L L L L L G L R L>
      SEAP SIGNAL PEPTIDE____>
      60      70      80      90      100
CAGCTGAGCCTGGGCCACGGCGAGGGCACCTTCACCAGCGACGTGAGCAG
GTCGACTCGGACCCGGTGCCGCTCCCGTGGAAGTGGTCGCTGCACTCGTC
  Q L S L G>
  SEAP SIG____>H G E G T F T S D V S S
      GLP-1GLY8____>
      110      120      130      140      150
CTACCTGGAGGGCCAGGCCGCAAGGAGTTCATCGCCTGGCTGGTGAAGG
GATGGACCTCCCGGTCCGGCGGTTCTCAAGTAGCGGACCGACCACTTCC
  Y L E G Q A A K E F I A W L V K>
      GLP-1GLY8____>
GCCGCGGC
CGGCGCCG
G R G>
____>

```

Figure 1

Exendin-4.GLP-1Gly8

```

      10      20      30      40      50
GAATTCCGCCCCACCATGAAGATCATCCTGTGGCTGTGTGTGTTCCGGCCTG
CTTAAGGCGGGTGGTACTTCTAGTAGGACACCGACACACACAAGCCGGAC
      M K I I L W L C V F G L>
      PROEXENDIN>

      60      70      80      90     100
TTCCTGGCCACCCTGTTCCCCATCAGCTGGCAGATGCCCCTGGAGTCCGG
AAGGACCGGTGGGACAAGGGGTAGTCGACCGTCTACGGGCACCTCAGGCC
  F L A T L F P I S W Q M P V E S G>
      PROEXENDIN>

     110     120     130     140     150
CCTGTCCTCCGAGGACTCCGCCAGCTCCGAGAGCTTCGCCAAGCGCATCA
GGACAGGAGGCTCCTGAGGCGGTGAGGCTCTCGAAGCGGTTTCGCGTAGT
  L S S E D S A S S E S F A K R I>
      PROEXENDIN>


     160     170     180     190     200
AGCGCCACGGCGAGGGCACCTTCAACGAGCGACGTGAGCAGCTACCTGGAG
TCGCGGTGCCGCTCCCGTGGAAGTGGTCGCTGCACTCGTCGATGGACCTC
  H G E G T F T S D V S S Y L E>
      GLP-1 GLY-8>
K R>
____>

     210     220     230     240     250
GGCCAGGCCGCGCAAGGAGTTCATCGCCTGGCTGGTGAAGGGCCGCGGCTG
CCGGTCCGGCGGTTCTCAAGTAGCGGACCGACCACTTCCCGGCGCCGAC
  G Q A A K E F I A W L V K G R G>
      GLP-1 GLY-8>

```

Figure 2

Helodermin.GLP-1Gly8

```

      10      20      30      40      50
GAATTCCGCCCCACCATGAAGAGCATCCTGTGGCTGTGTGTGTTTGGCCTG
CTTAAGGCGGGTGGTACTTCTCGTAGGACACCGACACACAAACCGGAC
      M K S I L W L C V F G L>
      PRO-HELODERMIN>

      60      70      80      90     100
      *
CTGATTGCCACCCTGTTCCCTGTGAGCTGGCAGATGGCCATCAAGAGCAG
GACTAACGGTGGGACAAGGGACACTCGACCGTCTACCGGTAGTTCTCGTC
L I A T L F P V S W Q M A I K S R>
      PRO-HELODERMIN>

     110     120     130     140     150
ACTGTCCTCTGAGGACTCTGAGACAGACCAGAGACTGAAGCGCATCAAGC
TGACAGGAGACTCCTGAGACTCTGTCTGGTCTCTGACTTCGCGTAGTTCC
L S S E D S E T D Q R L K R I K>
      PRO-HELODERMIN>

     160     170     180     190     200
      *
GCCACGGCGAGGGCACCTTCACCAGCGACGTGAGCAGCTACCTGGAGGGC
CGGTGCCGCTCCCGTGGAAGTGGTCGCTGCACTCGTCGATGGACCTCCCG
R>
      H G E G T F T S D V S S Y L E G>
      PRODUCT=GLP-1>

     210     220     230     240
CAGGCCGCCAAGGAGTTCATCGCCTGGCTGGTGAAGGGCCGCGGC
GTCCGGCGGTTCCTCAAGTAGCGGACCGACCACTTCCCGGCGCCG

```

```

Q A A K E F I A W L V K G R G>
      PRODUCT=GLP-1>

```

Figure 3

GIP.GLP-1Gly8

```

      10      20      30      40      50
GAATTCCGCCCACCATGGTGGCCACCAAGACCTTTGCCCTGCTGCTCCTG
CTTAAGGCGGGTGGTACCACCGGTGGTTCTGGAAACGGGACGACGAGGAC
      M V A T K T F A L L L L>
      PRO-GIP>

      60      70      80      90      100
      *
AGCCTCTTCCTGGCTGTGGGACTGGGCGAGAAGAAGGAAGGCCACTTTCAG
TCGGAGAAGGACCGACACCCTGACCCGCTCTTCTTCCTTCCGGTGAAGTC
S L F L A V G L G E K K E G H F S>
      PRO-GIP>

      110     120     130     140     150
CGCCCTGCCCAGCCTGCCAGTGGGCAGCCATGCCAAGGTGAGCTCCCCAC
GCGGGACGGGTCCGACGGTCACCCGTCGGTACGGTTCCACTCGAGGGGTG
A L P S L P V G S H A K V S S P>
      PRO-GIP>

      160     170     180     190     200
      *
AGAAGCGCATCAAGCGCCACGGCGAGGGCACCTTCACCAGCGACGTGAGC
TCTTCGCGTAGTTTCGCGGTGCCGCTCCCGTGGAAGTGGTCGCTGCACTCG
Q K R I K R>
      PRO-GIP>H G E G T F T S D V S>
      GLP-1GLY8>

      210     220     230     240     250
AGCTACCTGGAGGGCCAGGCCGCAAGGAGTTCATCGCCTGGCTGGTGAA
TCGATGGACCTCCCGGTCCGGCGGTTCCTCAAGTAGCGGACCGACCACTT
S Y L E G Q A A K E F I A W L V K>
      GLP-1GLY8>

      260
GGGCCGCGGC
CCCGGCGCCG
G R G>
      >

```

Figure 4

IGF-I(furin).GLP-1Gly8

```

      10      20      30      40      50
GAATTCGCCCCACCATGGGCAAGATCAGCAGCCTGCCCCACCCAGCTGTTC
CTTAAGGCGGGTGGTACCCGTTCTAGTCGTCGGACGGGTGGGTGACAAAG

      M  G  K  I  S  S  L  P  T  Q  L  F >
      _____IGF1 1-48_____>

      60      70      80      90      100
AAGTGCTGCTTTTGTGACTTCCTGAAGGTGAAGATGCACACCATGAGCTC
TTCACGACGAAAACACTGAAGGACTTCCACTTCTACGTGTGGTACTCGAG
K  C  C  F  C  D  F  L  K  V  K  M  H  T  M  S  S >
      _____IGF1 1-48_____>

      110     120     130     140     150
CAGCCACCTGTTCTACCTGGCCCTGTGCCTGCTGACCTTCACCAGCTCCG
GTCGGTGGACAAGATGGACCGGGACACGGACGACTGGAAGTGGTCGAGGC
S  H  L  F  Y  L  A  L  C  L  L  T  F  T  S  S >
      _____IGF1 1-48_____>

      160     170     180     190     200
CCACAGCCAAGCGCATCAAGCGCCACGGCGAGGGCACCTTCACCAGCGAC
GGTGTGCGTTGCGGTAGTTCGCGGTGCCGCTCCCGTGGAAGTGGTCGCTG
A  T  A >
      _____>K  R  I  K  R>
      _____FURIN CL_____>H  G  E  G  T  F  T  S  D>
      _____PRODUCT=GLP-1_____>

      210     220     230     240     250
GTGAGCAGCTACCTGGAGGGCCAGGCCGCCAAGGAGTTTCATCGCCTGGCT
CACTCGTCGATGGACCTCCCGGTCCGGCGGTTCTCAAGTAGCGGACCGA
V  S  S  Y  L  E  G  Q  A  A  K  E  F  I  A  W  L >
      _____PRODUCT=GLP-1_____>

      260
GGTGAAGGGCCGCGGC
CCACTTCCCGGCGCCG
V  K  G  R  G >
      _____PRODUCT=_____>
```

Figure 5

IGF-I.GLP-1Gly8

```

      10      20      30      40      50
GAATTCCGCCCCACCATGGGCAAGATCAGCAGCCTGCCCCACCCAGCTGTTC
CTTAAGGCGGGTGGTACCCGTTCTAGTCGTCGGACGGGTGGGTGACACAAG
      M G K I S S L P T Q L F>
      IGF-I SIGNAL PEPTIDE_____>

      60      70      80      90      100
AAGTGCTGCTTTTGTGACTTCCTGAAGGTGAAGATGCACACCATGAGCTC
*

TTCACGACGAAAACACTGAAGGACTTCCACTTCTACGTGTGGTACTCGAG
K C C F C D F L K V K M H T M S S>
      IGF-I SIGNAL PEPTIDE_____>

      110      120      130      140      150
CAGCCACCTGTTTCTACCTGGCCCTGTGCCTGCTGACCTTCACCAGCTCCG
GTCGGTGGACAAGATGGACCGGGACACGGACGACTGGAAGTGGTTCGAGGC
S H L F Y L A L C L L T F T S S>
      IGF-I SIGNAL PEPTIDE_____>

      160      170      180      190      200
CCACAGCCCCACGGCGAGGGCACCTTCACCAGCGACGTGAGCAGCTACCTG
GGTGTGCGGTGCCGCTCCCGTGGAAGTGGTCGCTGCACTCCTCGATGGAC
A T A>
      >H G E G T F T S D V S S Y L>
      GLP-1GLY8_____>

      210      220      230      240      250
GAGGGCCAGGCCGCCAAGGAGTTCATCGCCTGGCTGGTGAAGGGCCGCGGC
CTCCCGGTCCGGCGGTTCTCAAGTAGCGGACCGACCACTTCCCGGCGCCG
E G Q A A K E F I A W L V K G R G>
      GLP-1GLY8_____>

```

Figure 6

Preproglucagon.GLP-1GLY8

```

      10      20      30      40      50
GAATTCCGCCCACCATGAAAAGCATTTACTTTGTGGCTGGGCTGTTTGTG
CTTAAGGCGGGTGGTACTTTTCGTAAATGAAACACCGACCCGACAAACAC
      M K S I Y F V A G L F V>
      _____GLUCAGON SIGNAL PEPTIDE_____>

      60      70      80      90      100
ATGCTGGTGCAAGGCAGCTGGCAACACGGCGAGGGCACCTTCACCAGCGA
TACGACCACGTTCCGTCGACCGTTGTGCCGCTCCCGTGGAAGTGGTCGCT
      M L V Q G S W Q>
      _____GLUCAGON SIGNAL P_____>H G E G T F T S D>
      _____GLP-1GLY8_____>

      110      120      130      140      150
CGTGAGCAGCTACCTGGAGGGCCAGGCCGCAAGGAGTTCATCGCCTGGC
GCACTCGTCGATGGACCTCCCGGTCCGGCGGTTTCCTCAAGTAGCGGACCG
      V S S Y L E G Q A A K E F I A W>
      _____GLP-1GLY8_____>

      160
TGGTGAAGGGCCCGCGGC

ACCACTTCCCGGCGCCG
L V K G R G>
      _____GLP-1GLY8_____>

```

Figure 7

Alpha-1 antitrypsin.GLP-1Gly8

```

      10      20      30      40      50
GAATTCCGCCCACCATGCCCTCTTCTGTCTCCTGGGGCATCCTCCTGCTG
CTTAAGGCGGGTGGTACGGGAGAAGACAGAGGACCCCGTAGGAGGACGAC
      M P S S V S W G I L L L>
      _____A1AT SIGNAL PEPTIDE_____>

      60      70      80      90      100
GCAGGCCTGTGCTGCCTGGTCCCTGTCTCCCTGGCTCACGGCGAGGGCAC
CGTCCGGACACGACGGACCAGGGACAGAGGGACCGAGTGCCGCTCCCGTG
A G L C C L V P V S L A>
      _____A1AT SIGNAL PEPTIDE_____>H G E G T>
      _____>

      110      120      130      140      150
CTTCACCAGCGACGTGAGCAGCTACCTGGAGGGCCAGGCCGCCAAGGAGT
GAAGTGGTCGCTGCACTCGTCGATGGACCTCCCGGTCCGGCGGTTCTCA
F T S D V S S Y L E G Q A A K E>
      _____GLP-1GLY8_____>

      160      170
TCATCGCCTGGCTGGTGAAGGGCCGCGGC
AGTAGCGGACCGACCACTTCCCGGCGCCG
F I A W L V K G R G>
      _____GLP-1GLY8_____>

```

Figure 8



Factor IX.GLP-1Gly8

```

      10      20      30      40      50
GAATTCCGCCCACCATGCAGAGAGTGAACATGATCATGGCAGAAATCCCCA
CTTAAGGCGGGTGGTACGTCTCTCACTTGTACTAGTACCGTCTTAGGGGT
      M  Q  R  V  N  M  I  M  A  E  S  P>
      PRO-FIX
      60      70      80      90      100
GGCCTGATCACCATCTGCCTCCTGGGATACCTCCTGTCTGCTGAGTGCAC
CCGGACTAGTGGTAGACGGAGGACCTATGGAGGACAGACGACTCACGTG
G  L  I  T  I  C  L  L  G  Y  L  L  S  A  E  C  T>
      PRO-FIX
      110     120     130     140     150
AGTGTTCCTGGACCATGAGAATGCCAACAAAGATTCTGAACAGACCCAAGA

TCACAAGGACCTGGTACTCTTACGGTTGTTCTAAGACTTGTCTGGGTTCT
V  F  L  D  H  E  N  A  N  K  I  L  N  R  P  K>
      PRO-FIX
      160     170     180     190     200
GGCATGGGGAGGGCACCTTCACCAGCGACGTGAGCAGCTACCTGGAGGGC
CCGTACCCCTCCCGTGGAAGTGGTCGCTGCACTCGTCGATGGACCTCCCG
R>
__>H  G  E  G  T  F  T  S  D  V  S  S  Y  L  E  G>
      GLP-1GLY-8
      210     220     230     240
CAGGCCGCCAAGGAGTTCATCGCCTGGCTGGTGAAGGGCCGCGGC
GTCCGGCGGTTCTCAAGTAGCGGACCGACCACTTCCCGGCGCCG
Q  A  A  K  E  F  I  A  W  L  V  K  G  R  G>
      GLP-1GLY-8

```

Figure 9

Exendin-4 (IGF-I) .GLP-1Gly8

```

      10      20      30      40      50
GAATTCCGCCCCACCATGAAGATCATCCTGTGGCTGTGTGTGTTCCGGCCTG
CTTAAGGCGGGTGGTACTTCTAGTAGGACACCGACACACACAAGCCGGAC
      M K I I L W L C V F G L>
      PRO-EXENDIN-4>

      60      70      80      90     100
TTCCTGGCCACCCTGTTCCCCATCAGCTGGCAGATGCCCGTGGAGTCCGG
AAGGACCGGTGGGACAAGGGGTAGTCGACCGTCTACGGGCACCTCAGGCC
F L A T L F P I S W Q M P V E S G>
      PRO-EXENDIN-4>

     110     120     130     140     150
CCTGTCCTCCGAGGACTCCGCCAGCTCCGAGAGCCCTCTGAAGCCTGCCA
GGACAGGAGGCTCCTGAGGCGGTGAGGCTCTCGGGAGACTTCGGACGGT
L S S E D S A S S E S>
      PRO-EXENDIN-4>P L K P A>
      IGF-I PRO>

     160     170     180     190     200
AGTCTGCCAGACATGGAGAGGGGCACCTTCACATCTGACGTGAGCAGCTAC
TCAGACGGTCTGTACCTCTCCCGTGGGAAGTGTAGACTGCACTCGTTCGATG
      H G E G T F T S D V S S Y>
      GLP-1GLY8>

K S A R>
>

```

```

     210     220     230     240     250
CTGGAGGGCCAGGCCGCCAAGGAGTTCATCGCCTGGCTGGTGAAGGGCCGCGGC
GACCTCCCGGTCCGGCGGTTCCCTCAAGTAGCGGACCGACCACTTCCCGGCGCCG
L E G Q A A K E F I A W L V K G R G>
      GLP-1GLY8>

```

Figure 10

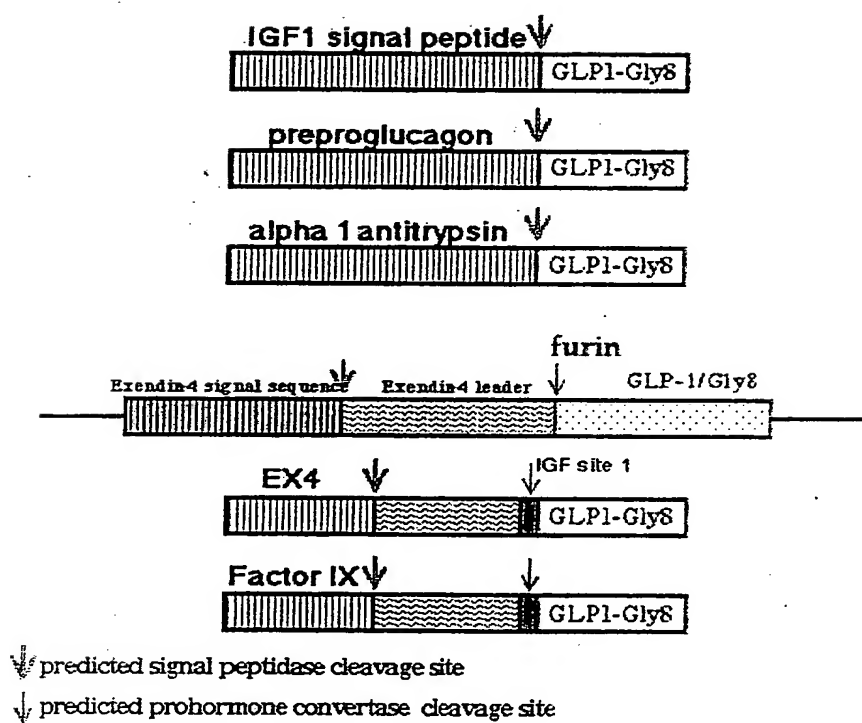


Figure 11

## GLP-1 Expression Levels in the Supernatant of Transfected 293 Cells

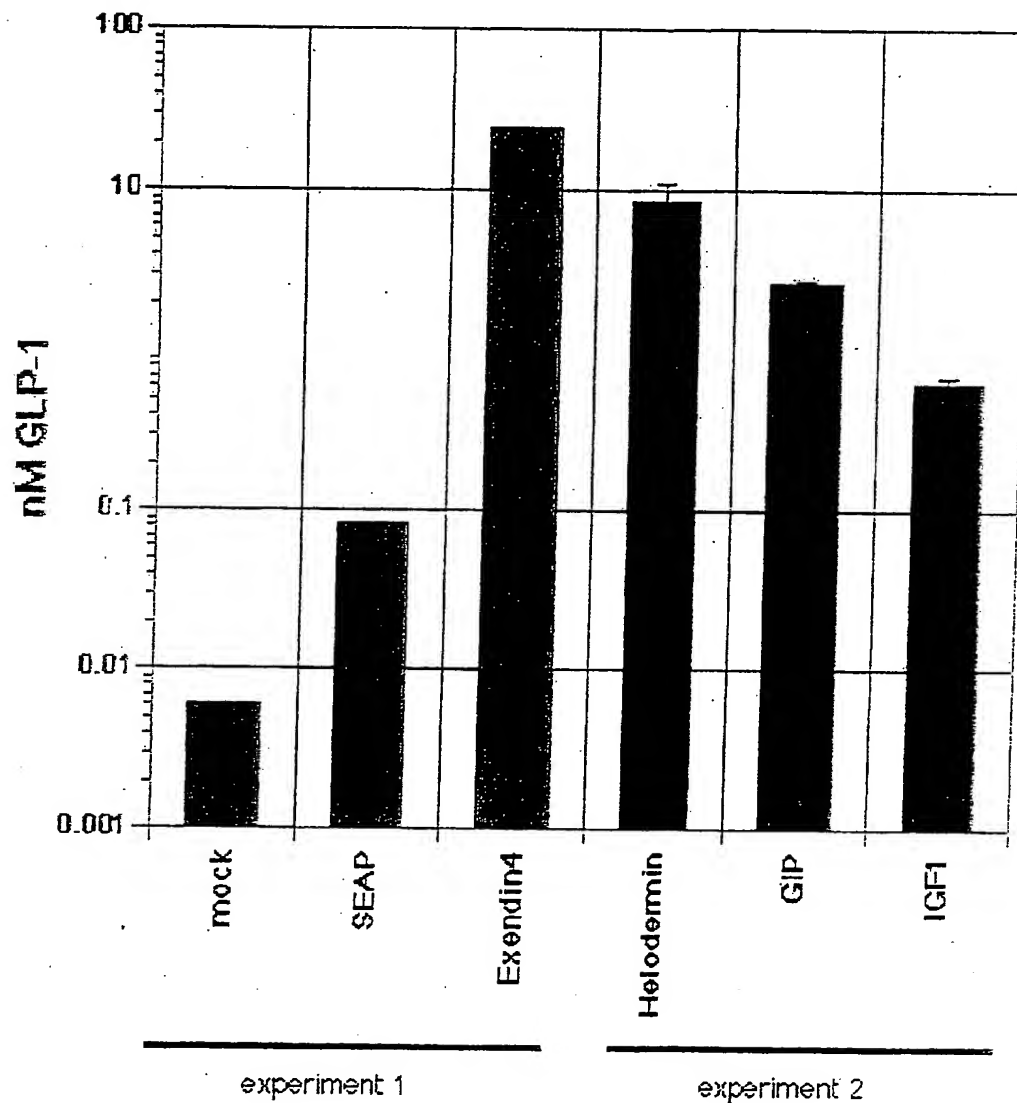


Figure 12

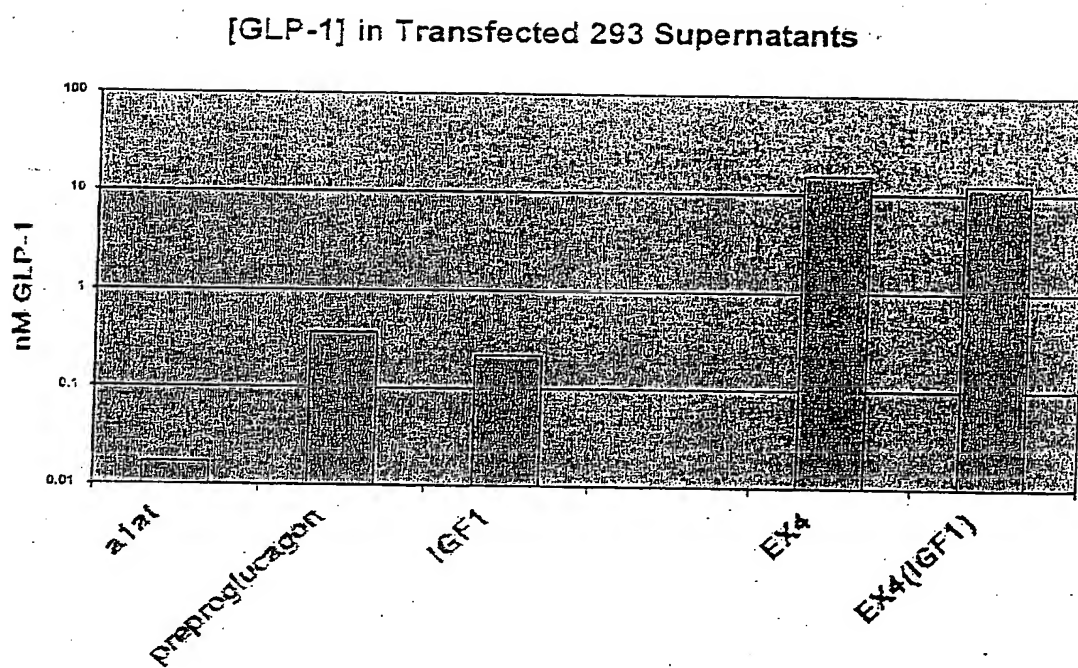


Figure 13

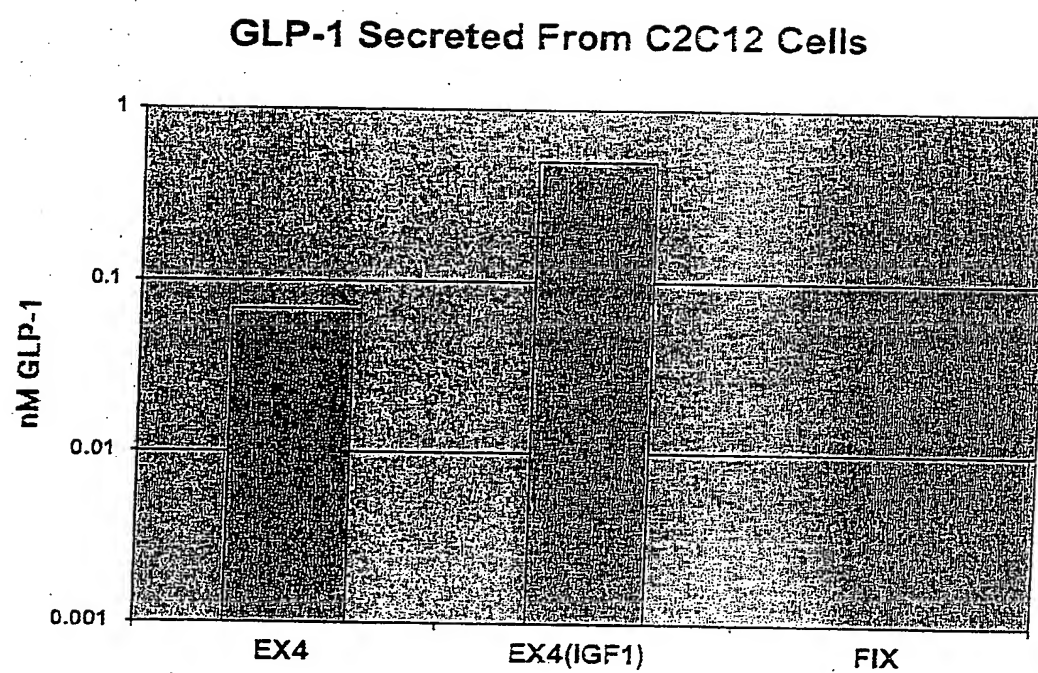


Figure 14

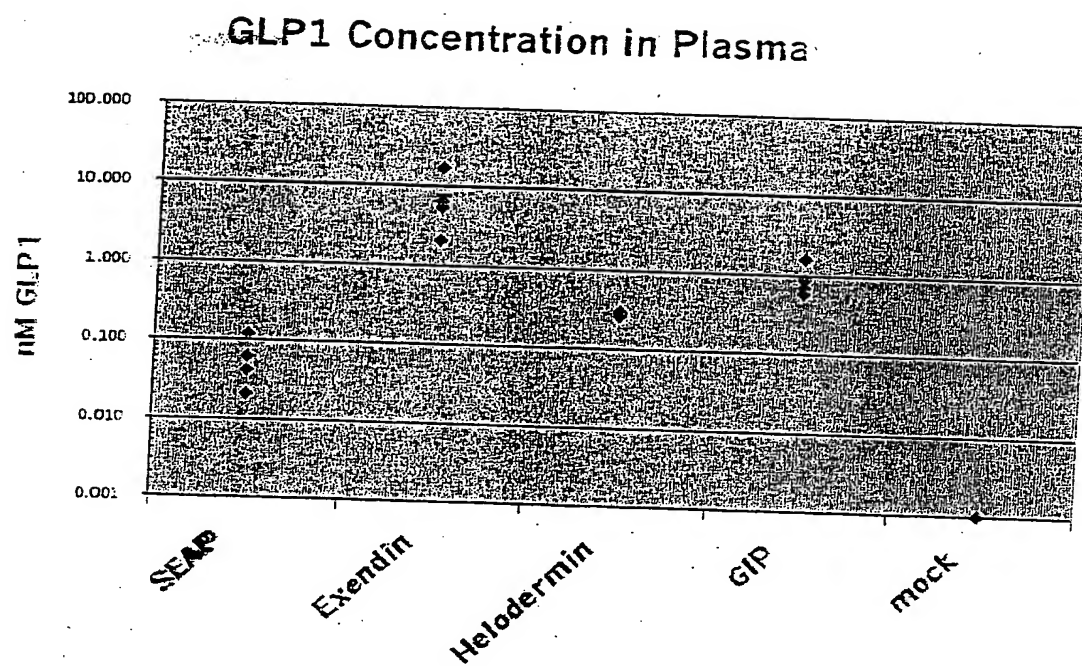


Figure 15

Blood Glucose Levels in db/db and Lean Mice Treated with a GLP-1 Expression Vector

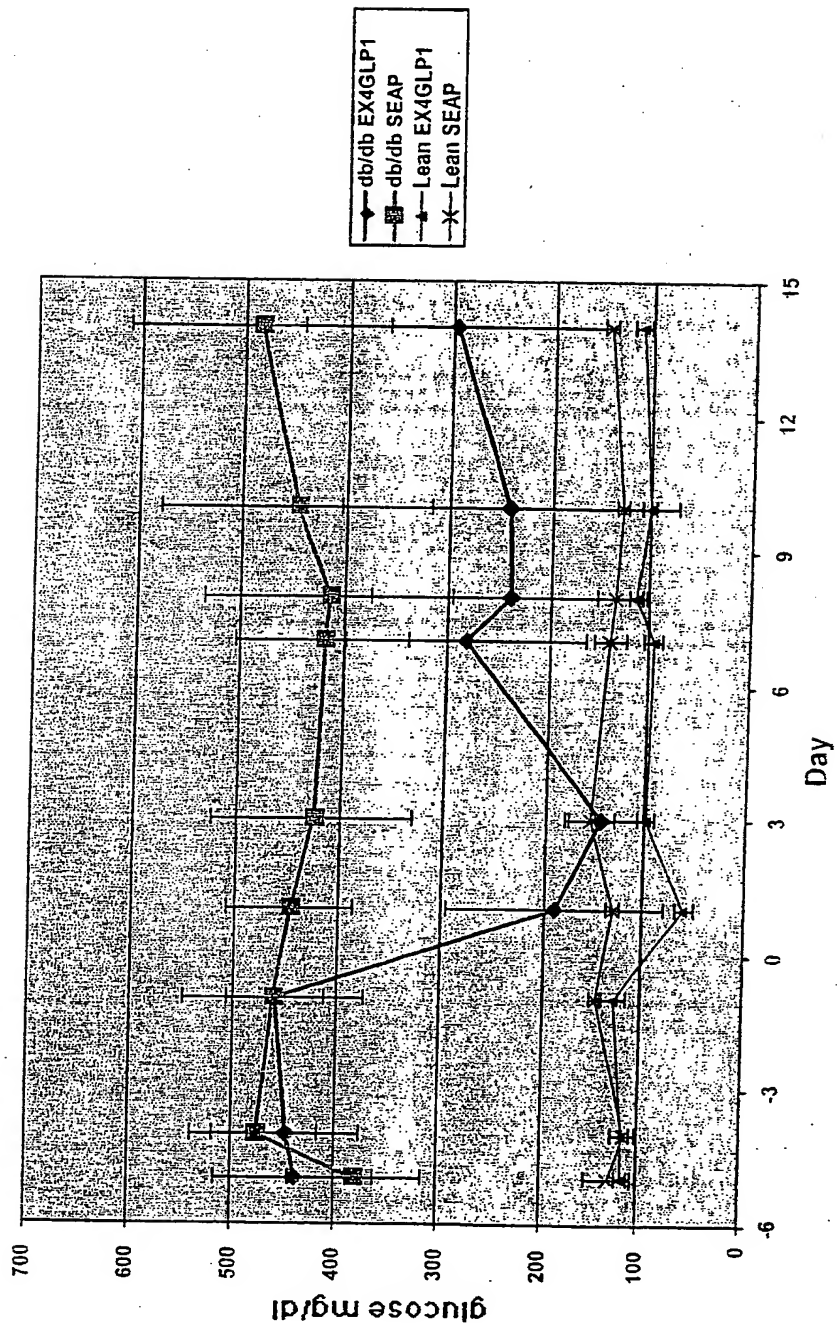


Figure 16



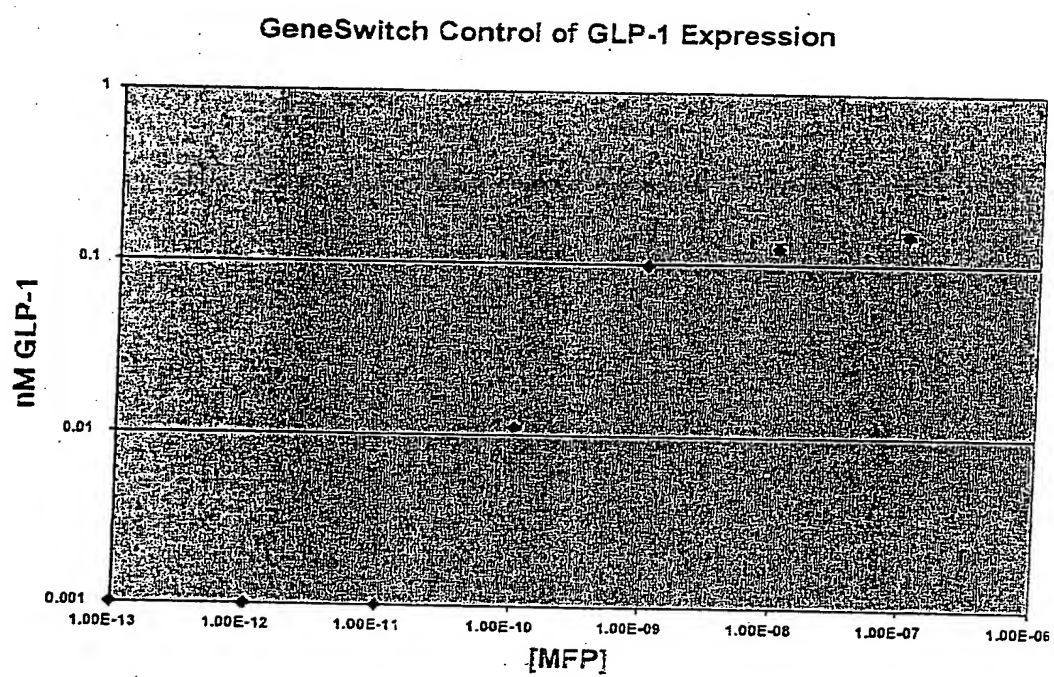


Figure 17

# Examples of Modified GLP-1

His<sup>7</sup>-Ala-Glu-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Val-Ser-Ser-Tyr-Leu<sup>20</sup>-Glu-Gly-Gln-Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-Ala<sup>30</sup>-Trp-Leu-Val-Lys (SEQ ID NO:23)

His<sup>7</sup>-Ala-Glu-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Val-Ser-Ser-Tyr-Leu<sup>20</sup>-Glu-Gly-Gln-Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-Ala<sup>30</sup>-Trp-Leu-Val-Lys-Gly<sup>35</sup> (SEQ ID NO: 24)

His<sup>7</sup>-Ala-Glu-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Val-Ser-Ser-Tyr-Leu<sup>20</sup>-Glu-Gly-Gln-Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-Ala<sup>30</sup>-Trp-Leu-Val-Lys-Gly<sup>35</sup>-Arg (SEQ ID NO:25)

His<sup>7</sup>-Val-Glu-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Val-Ser-Ser-Tyr-Leu<sup>20</sup>-Glu-Gly-Gln-Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-Ala<sup>30</sup>-Trp-Leu-Val-Lys-Gly<sup>35</sup>-Arg-Gly<sup>37</sup>-COOH (SEQ ID NO:26)

His<sup>7</sup>-Ala-Gln-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Val-Ser-Ser-Tyr-Leu<sup>20</sup>-Glu-Gly-Gln-Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-Ala<sup>30</sup>-Trp-Leu-Val-Lys-Gly<sup>35</sup>-Arg-Gly<sup>37</sup>-COOH (SEQ ID NO:27)

His<sup>7</sup>-Ala-Glu-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Thr-Ser-Lys-Tyr-Leu<sup>20</sup>-Glu-Gly-Gln-Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-Ala<sup>30</sup>-Trp-Leu-Val-Lys-Gly<sup>35</sup>-Arg-Gly<sup>37</sup> (SEQ ID NO:28)

His<sup>7</sup>-Ala-Glu-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Val-Ser-Lys-Tyr-Leu<sup>20</sup>-Glu-Gly-Gln-Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-Ala<sup>30</sup>-Trp-Leu-Val-Lys-Gly<sup>35</sup>-Arg-Gly<sup>37</sup>-COOH (SEQ ID NO:29)

His<sup>7</sup>-Ala-Glu-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Val-Ser-Ser-Tyr-Leu<sup>20</sup>-Glu-Gly-Gln-Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-D-Gln<sup>30</sup>-Trp-Leu-Val-Lys-Gly<sup>35</sup>-Arg-Gly<sup>37</sup>-COOH (SEQ ID NO:30)

Figure 18A

Asp-Glu-Phe-Glu-Arg-His<sup>7</sup>-Ala-Glu-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Val-Ser-Ser-Tyr-  
Leu<sup>20</sup>-Glu-Gly-Gln-Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-Ala<sup>30</sup>-Trp-Leu-Val-Lys-Gly<sup>35</sup>-Arg-Gly<sup>37</sup>-  
COOH (SEQ ID NO:31)

Glu-Phe-Glu-Arg-His<sup>7</sup>-Ala-Glu-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Val-Ser-Ser-Tyr-Leu<sup>20</sup>-  
Glu-Gly-Gln-Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-Ala<sup>30</sup>-Trp-Leu-Val-Lys-Gly<sup>35</sup>-Arg-Gly<sup>37</sup>-  
COOH (SEQ ID NO:32)

Arg-His<sup>7</sup>-Ala-Glu-Gly<sup>10</sup>-Thr-Phe-Thr-Ser-Asp<sup>15</sup>-Val-Ser-Ser-Tyr-Leu<sup>20</sup>-Glu-Gly-Gln-  
Ala-Ala<sup>25</sup>-Lys-Glu-Phe-Ile-Ala<sup>30</sup>-Trp-Leu-Val-Lys-Gly<sup>35</sup>-Arg-Gly<sup>37</sup>-COOH (SEQ ID  
NO:33)

**Figure 18B**

Figure 19

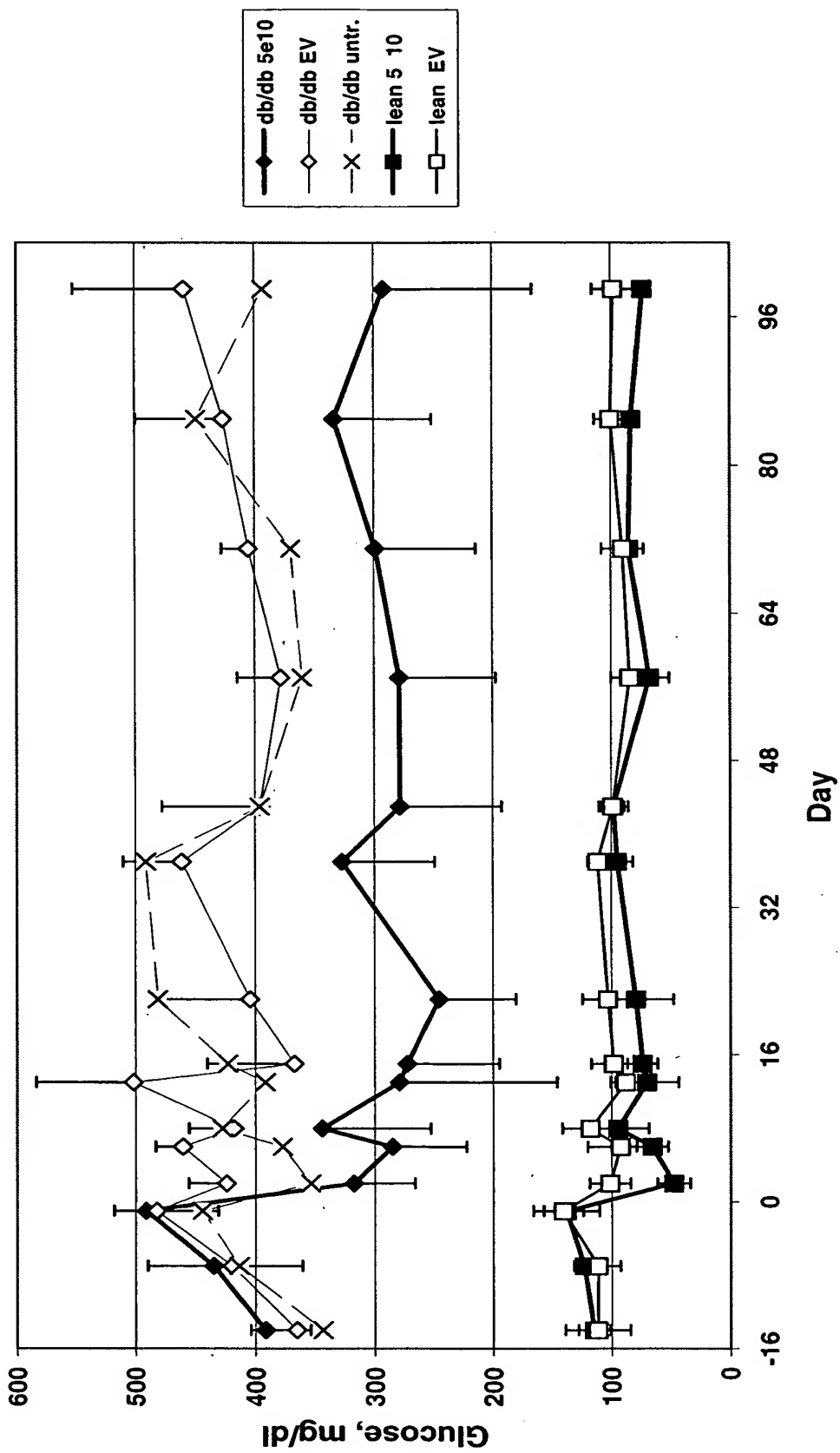




Figure 21

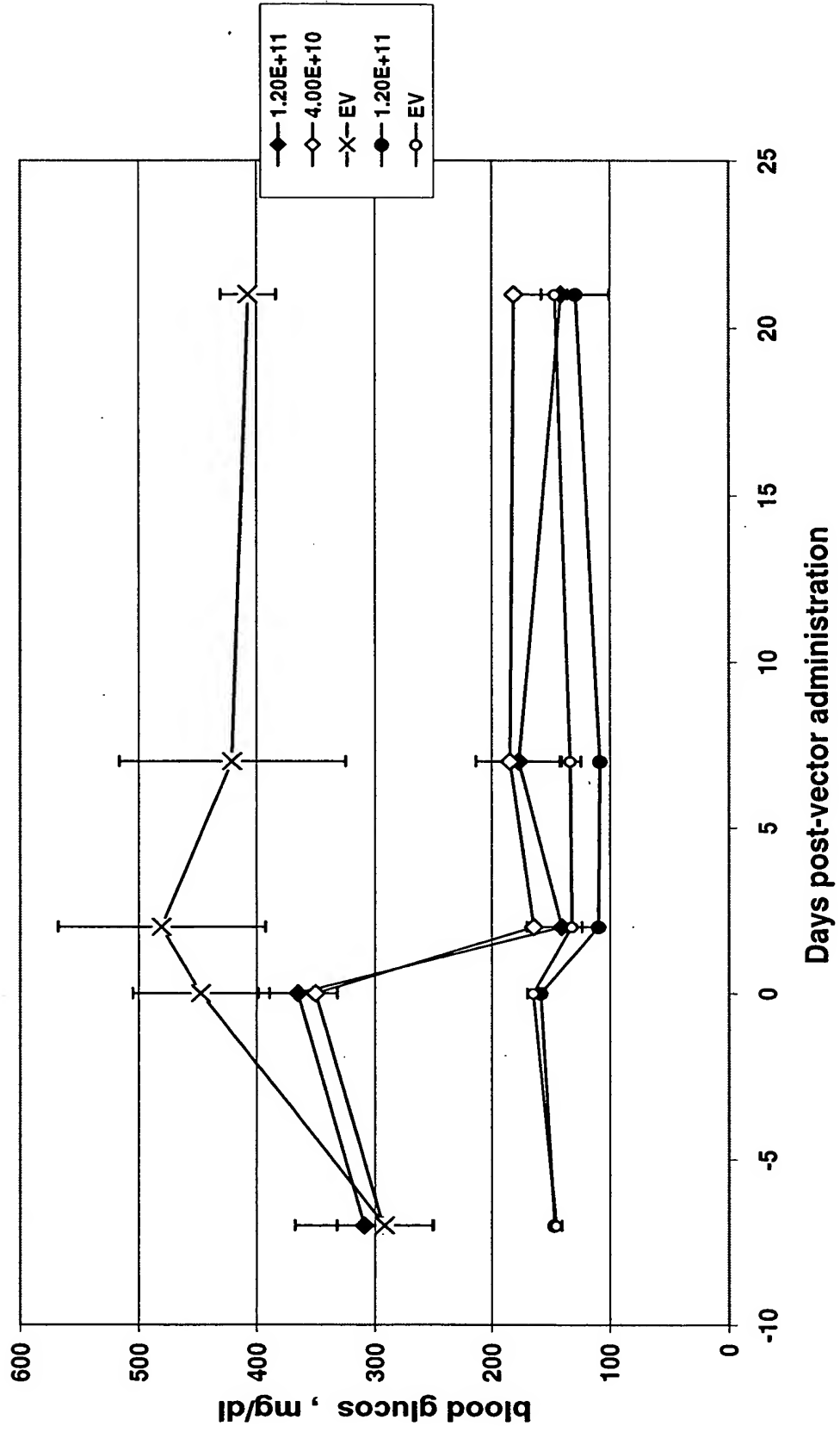


Figure 22

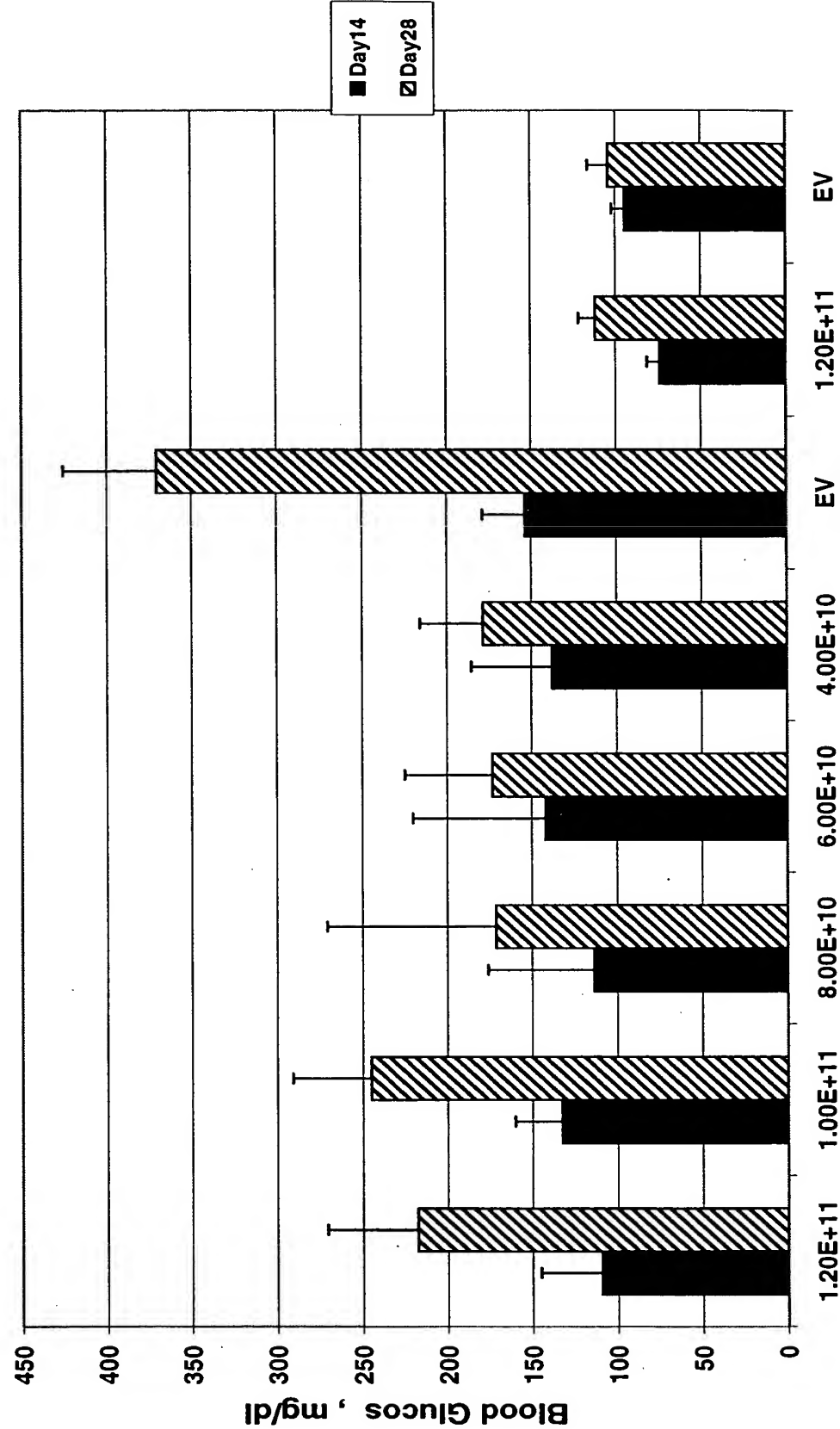


Figure 23

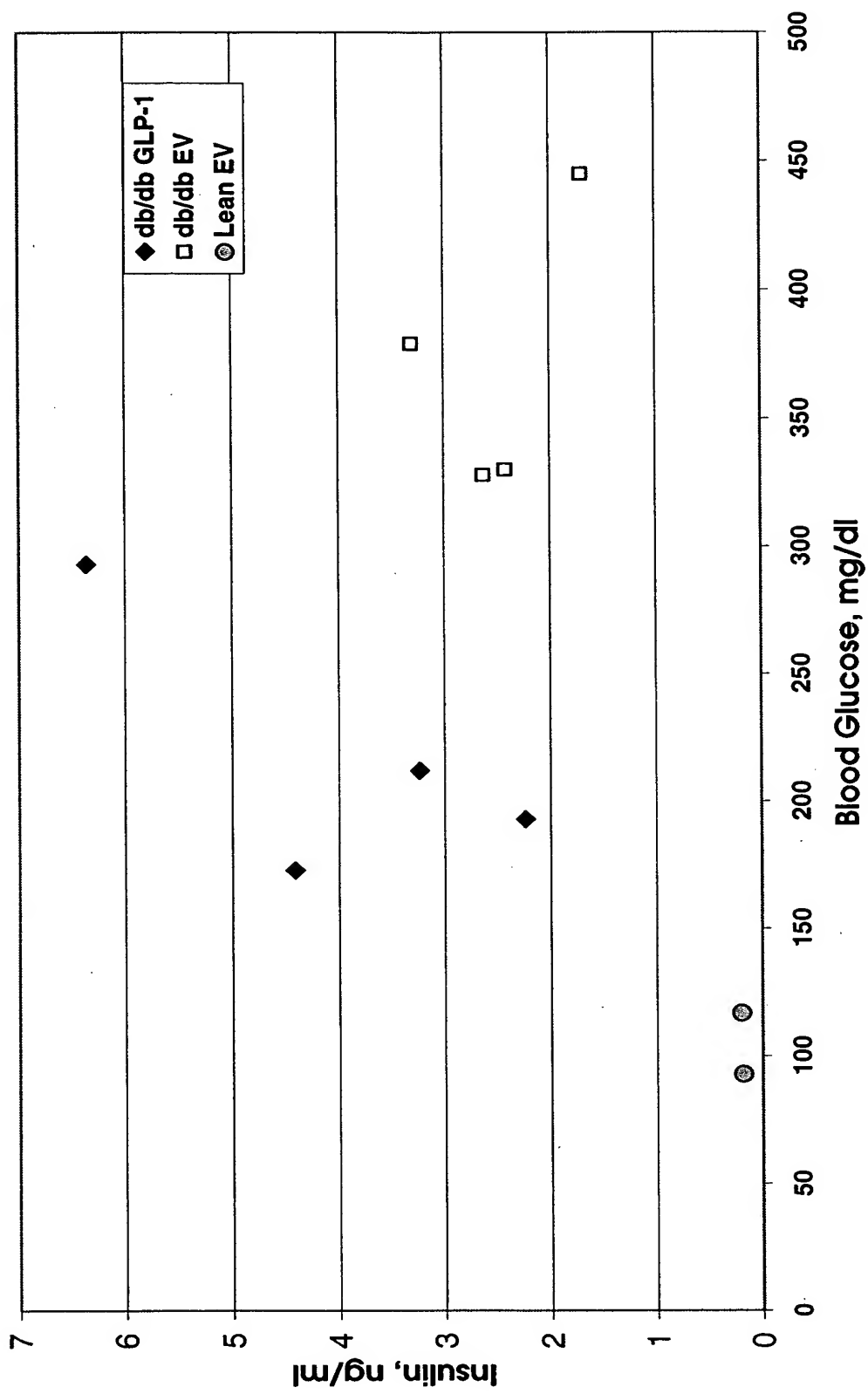




Figure 24

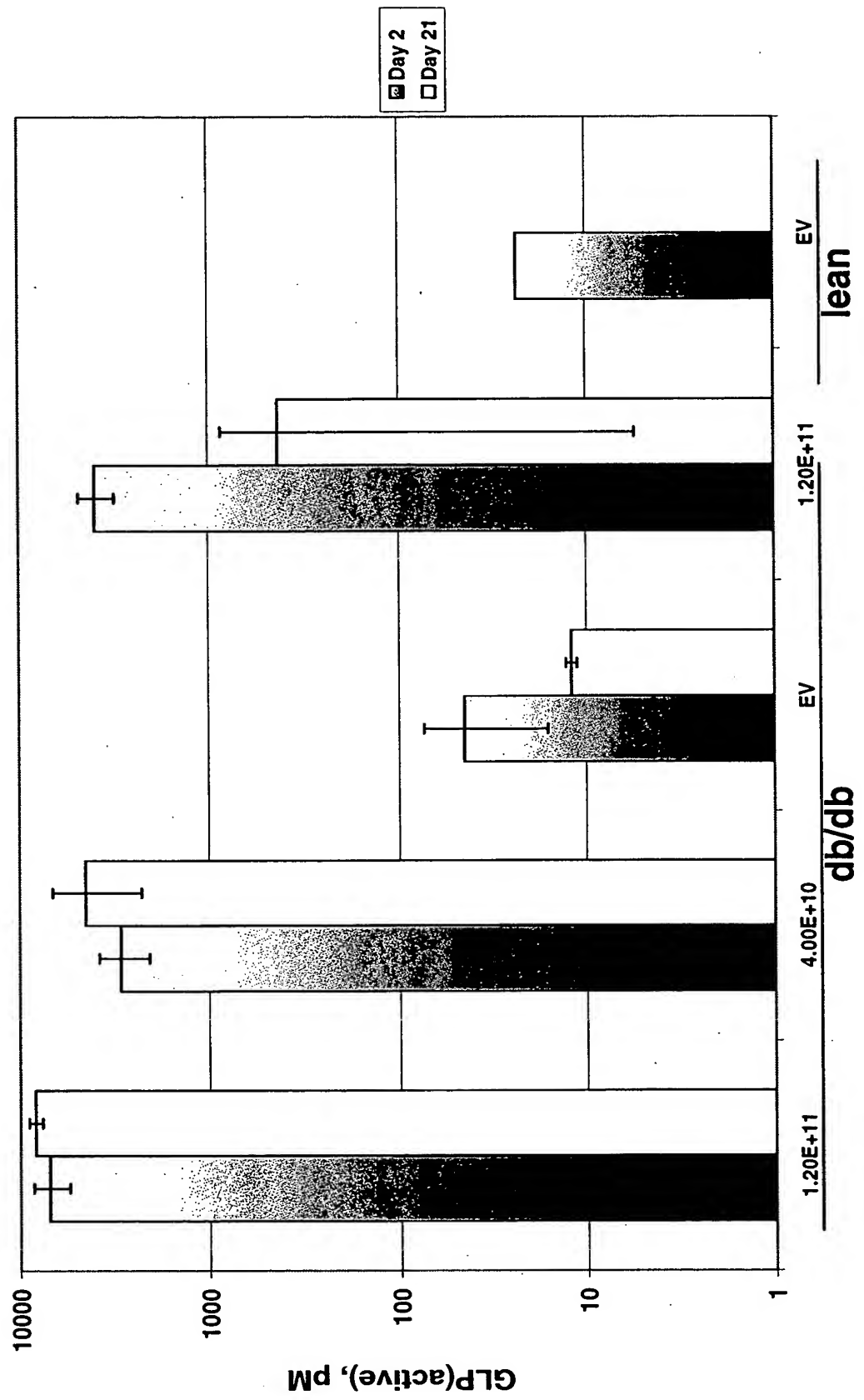


Figure 25

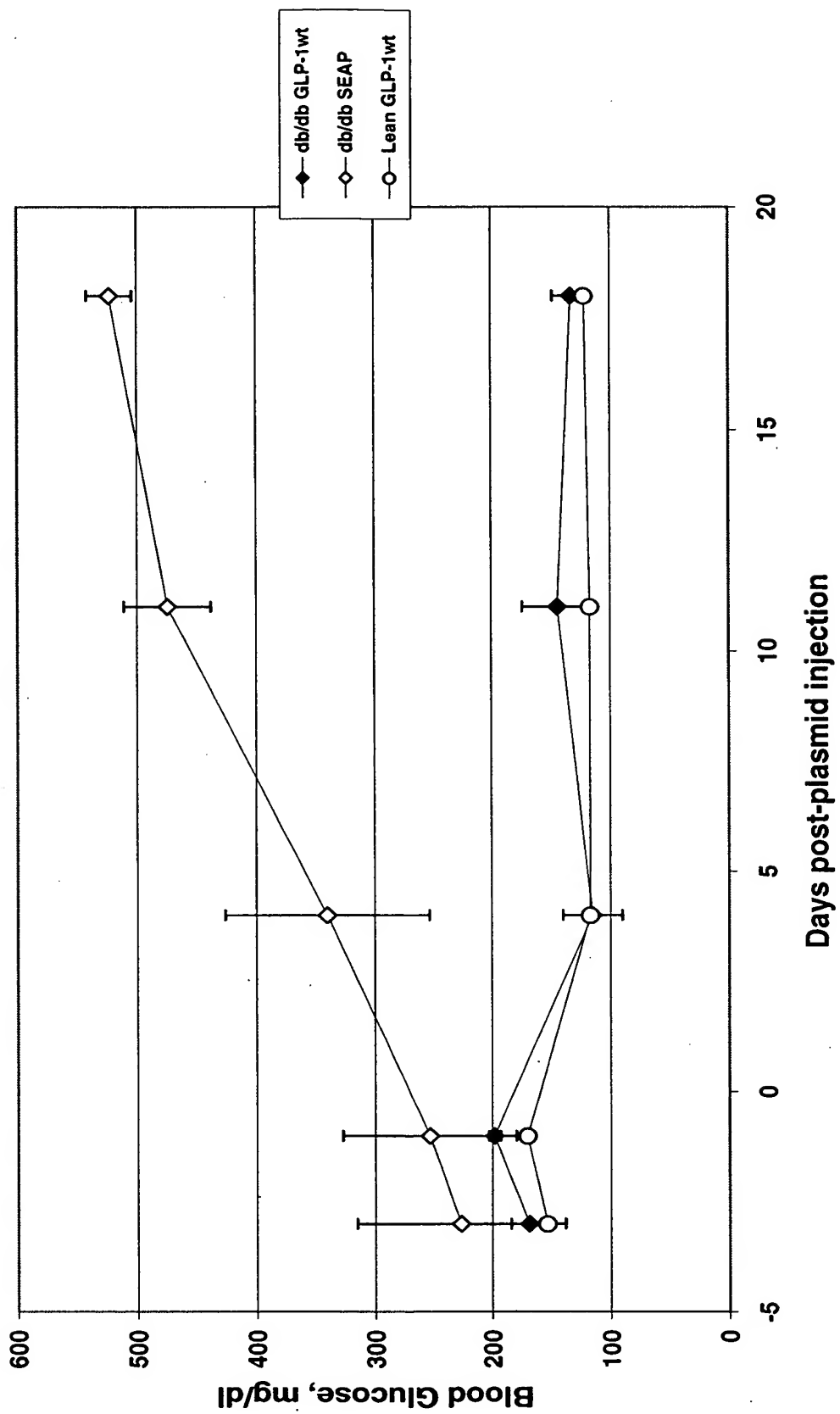


Figure 26

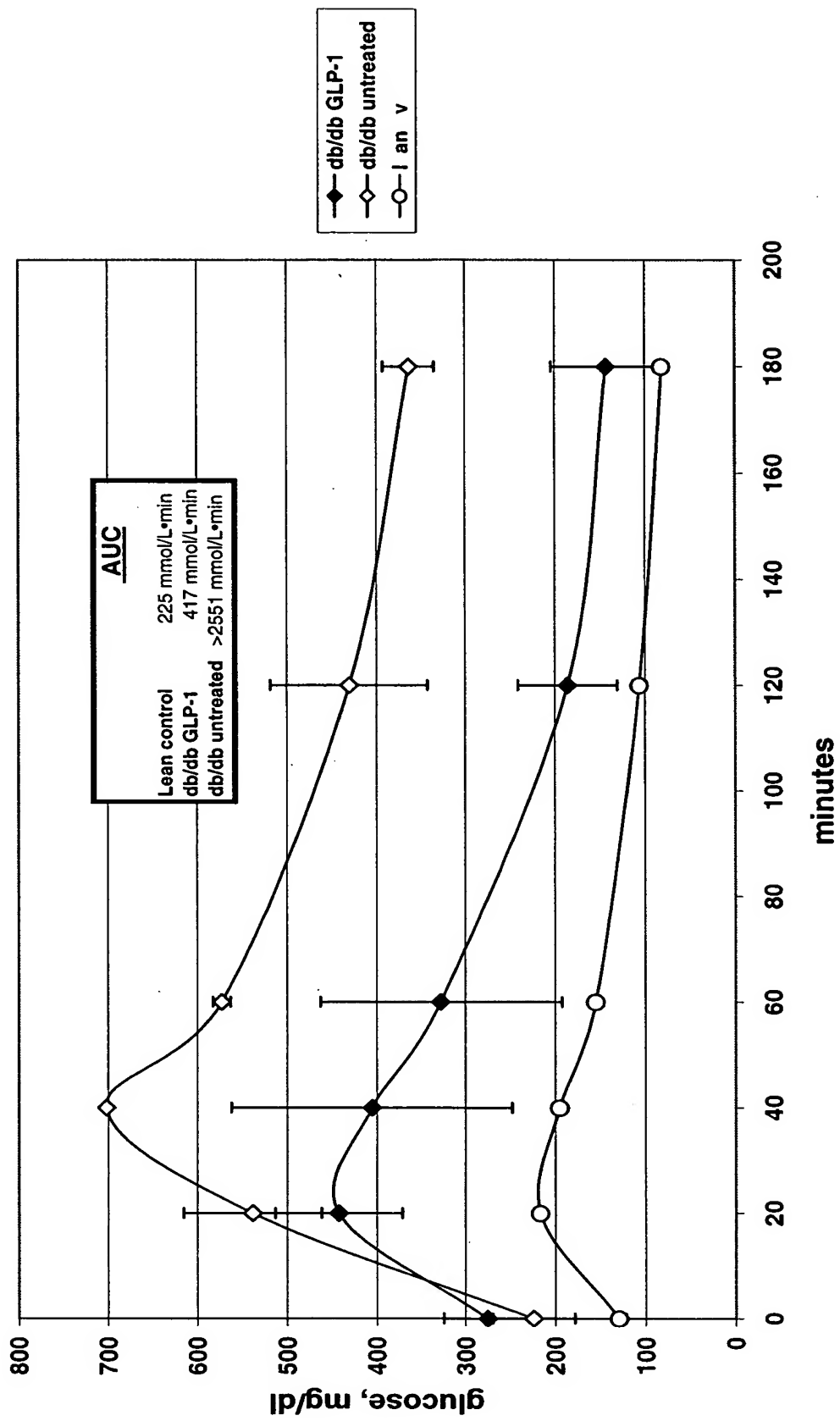


Figure 27

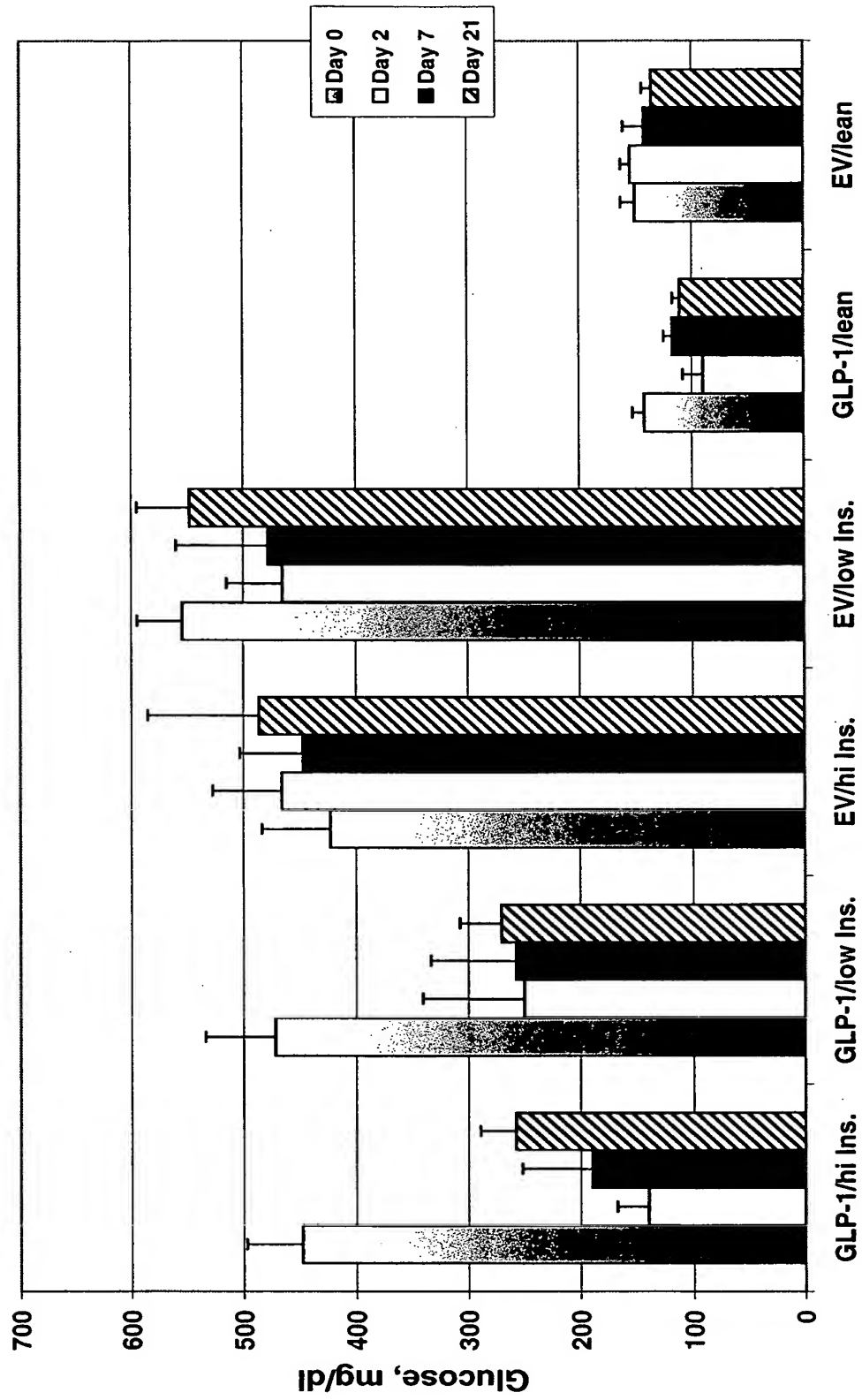


Figure 28

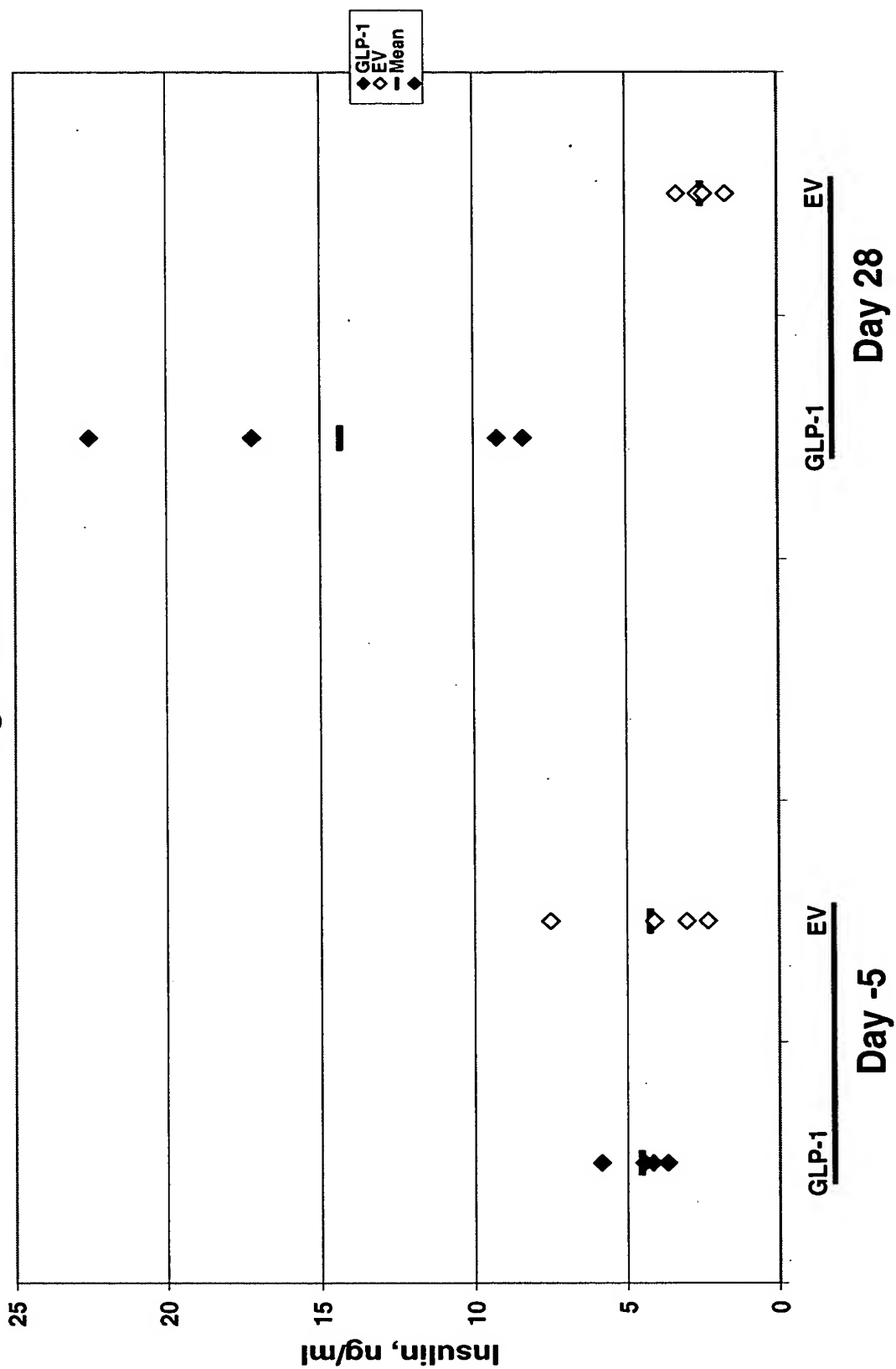


Figure 29

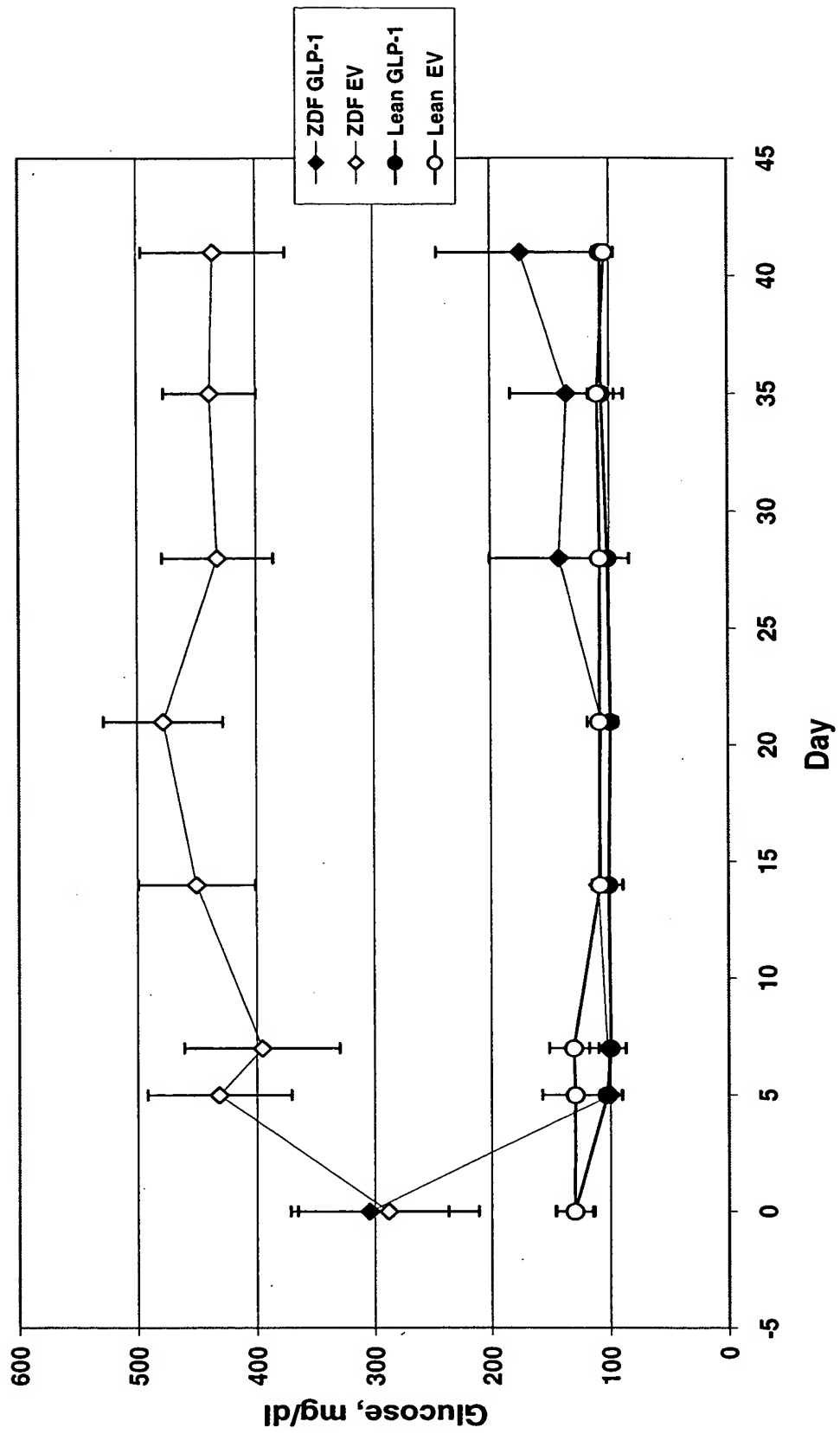


Figure 30

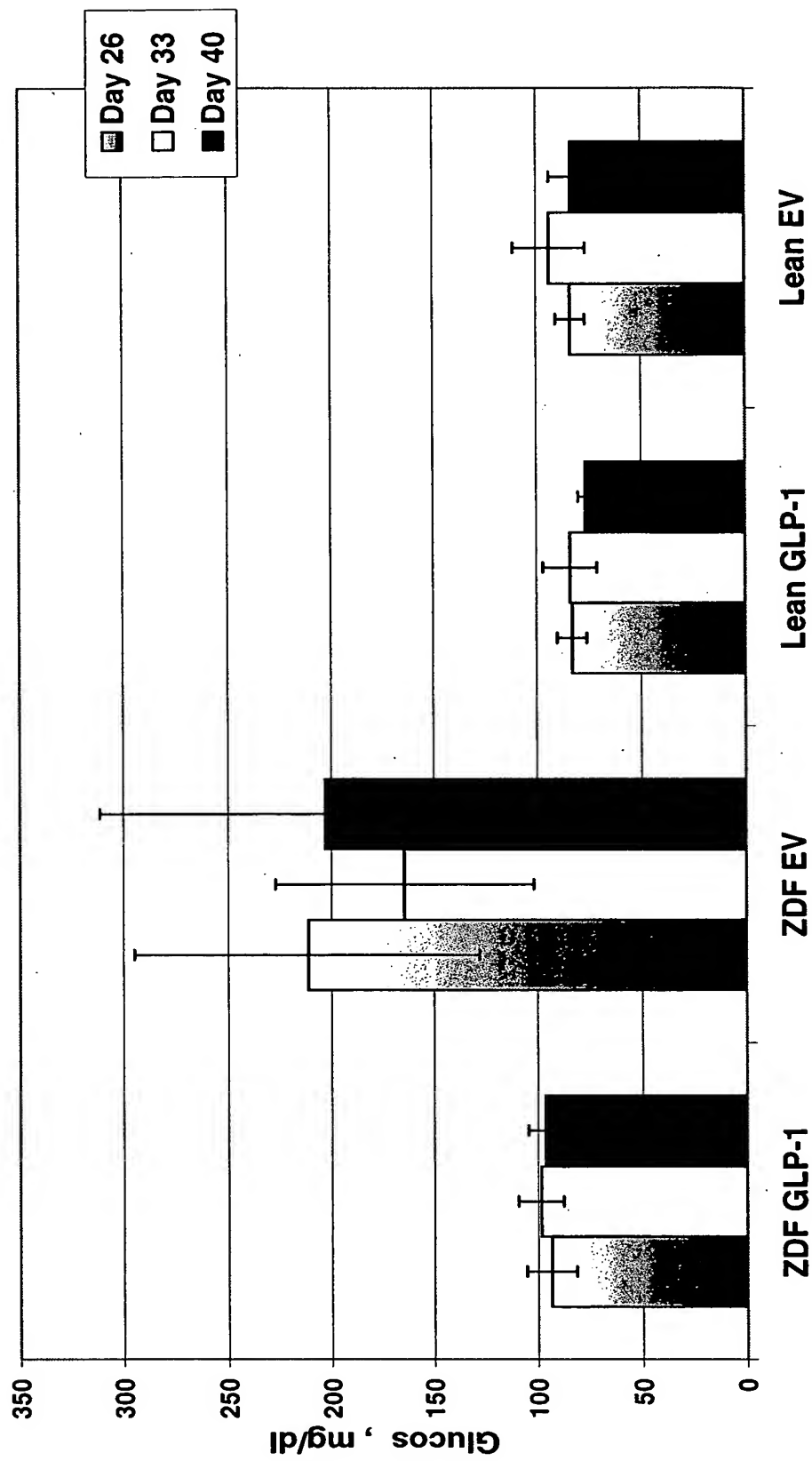


Figure 31

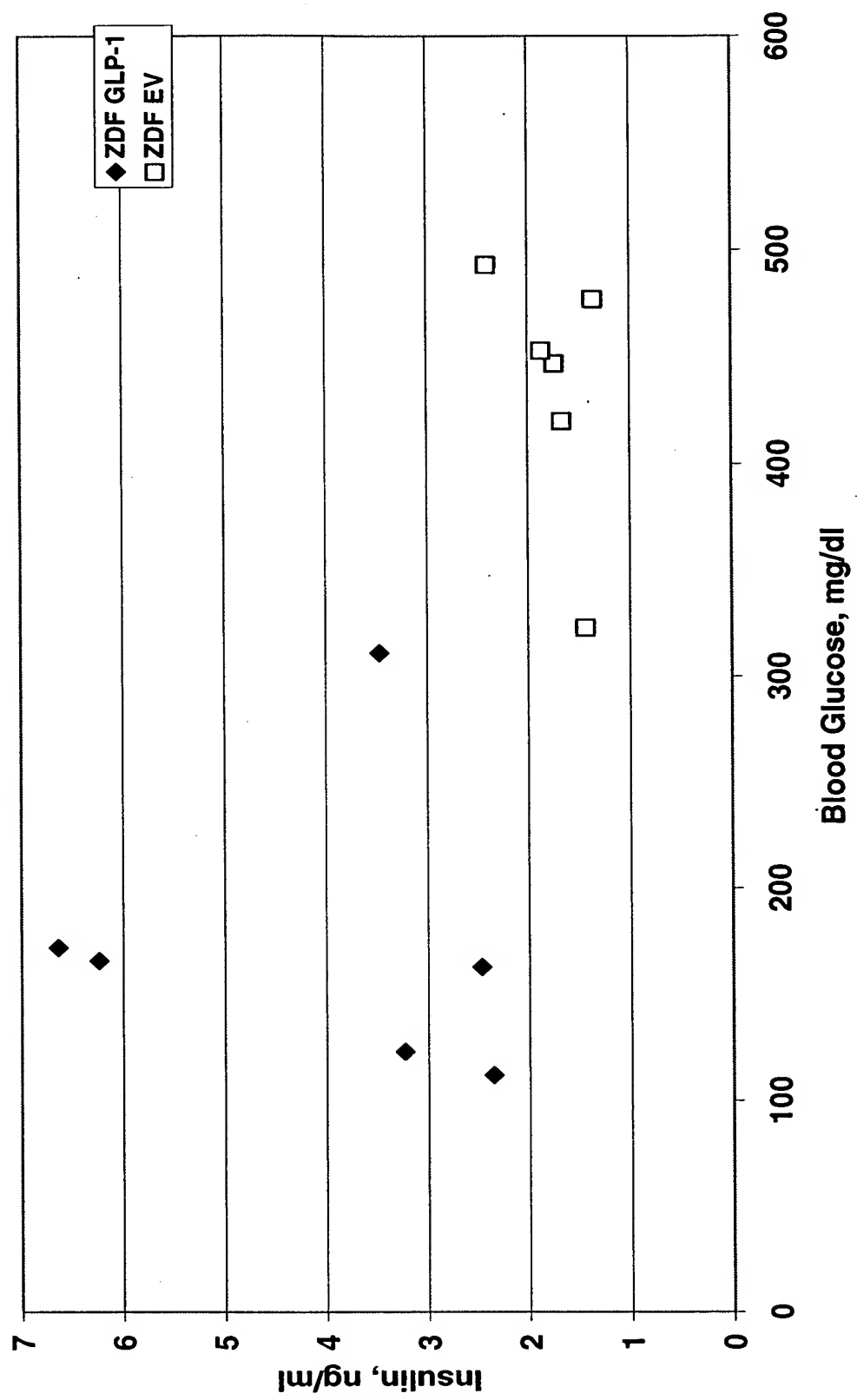
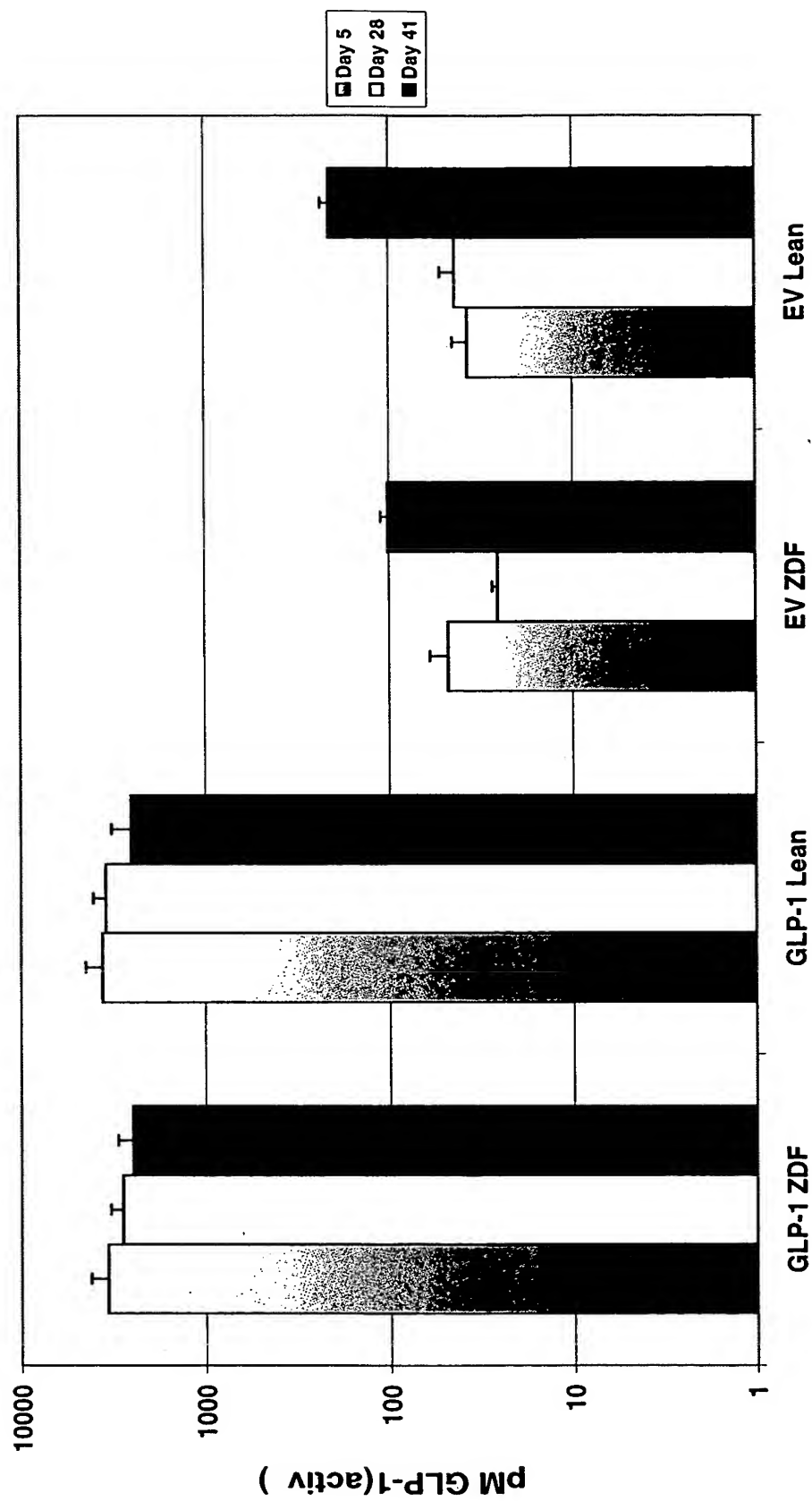




Figure 32



# Figure 33

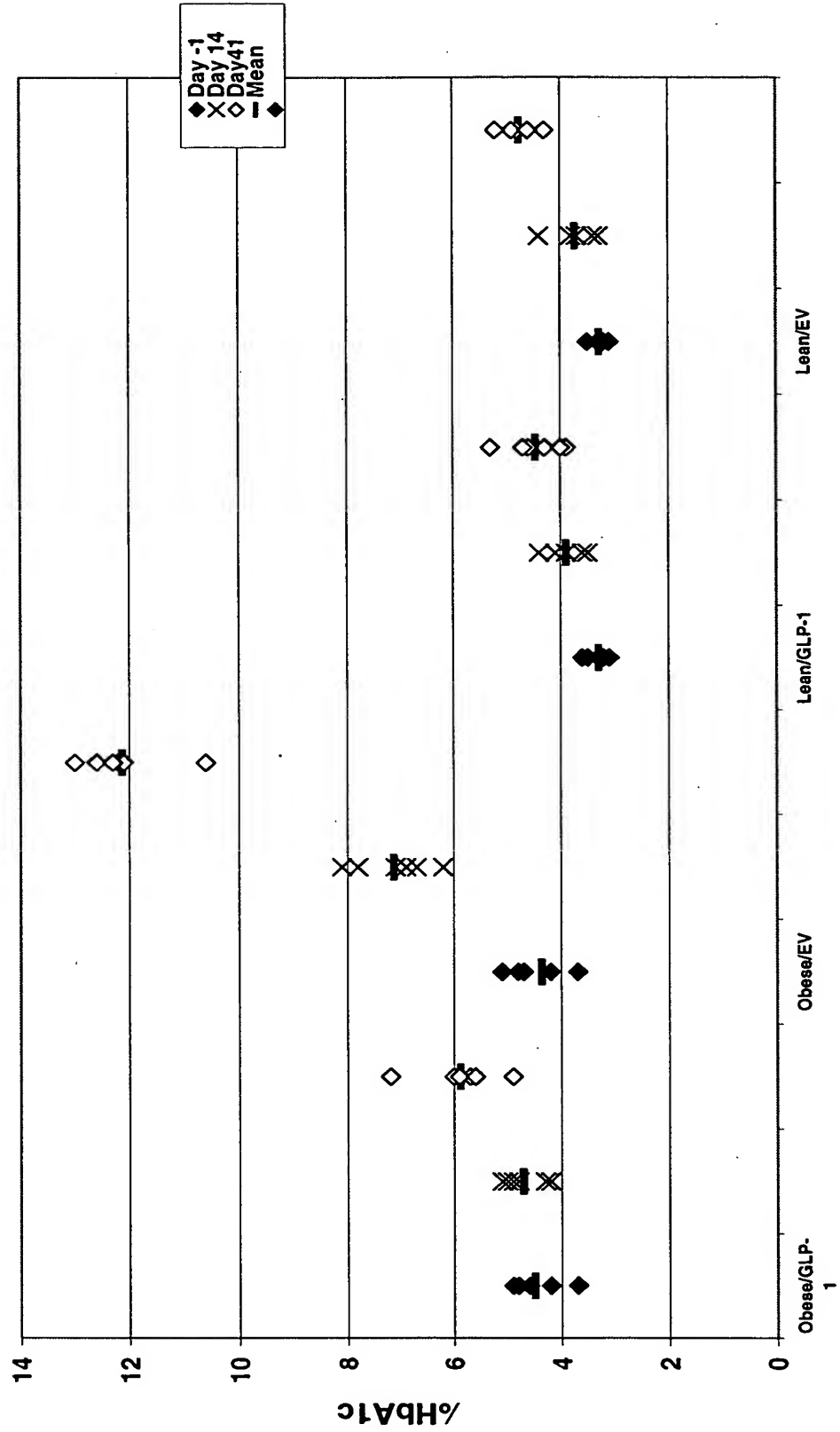


Figure 34

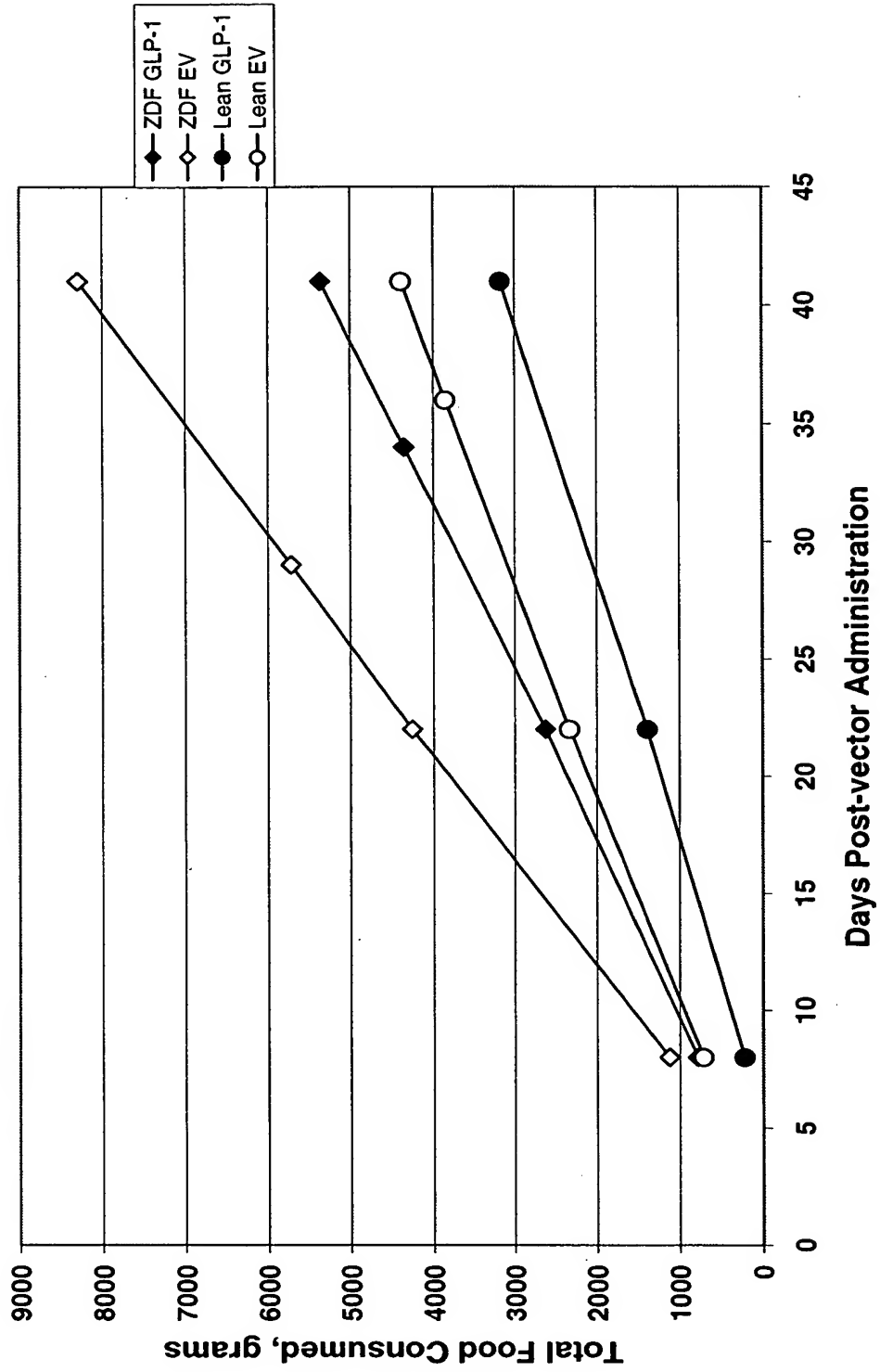


Figure 35

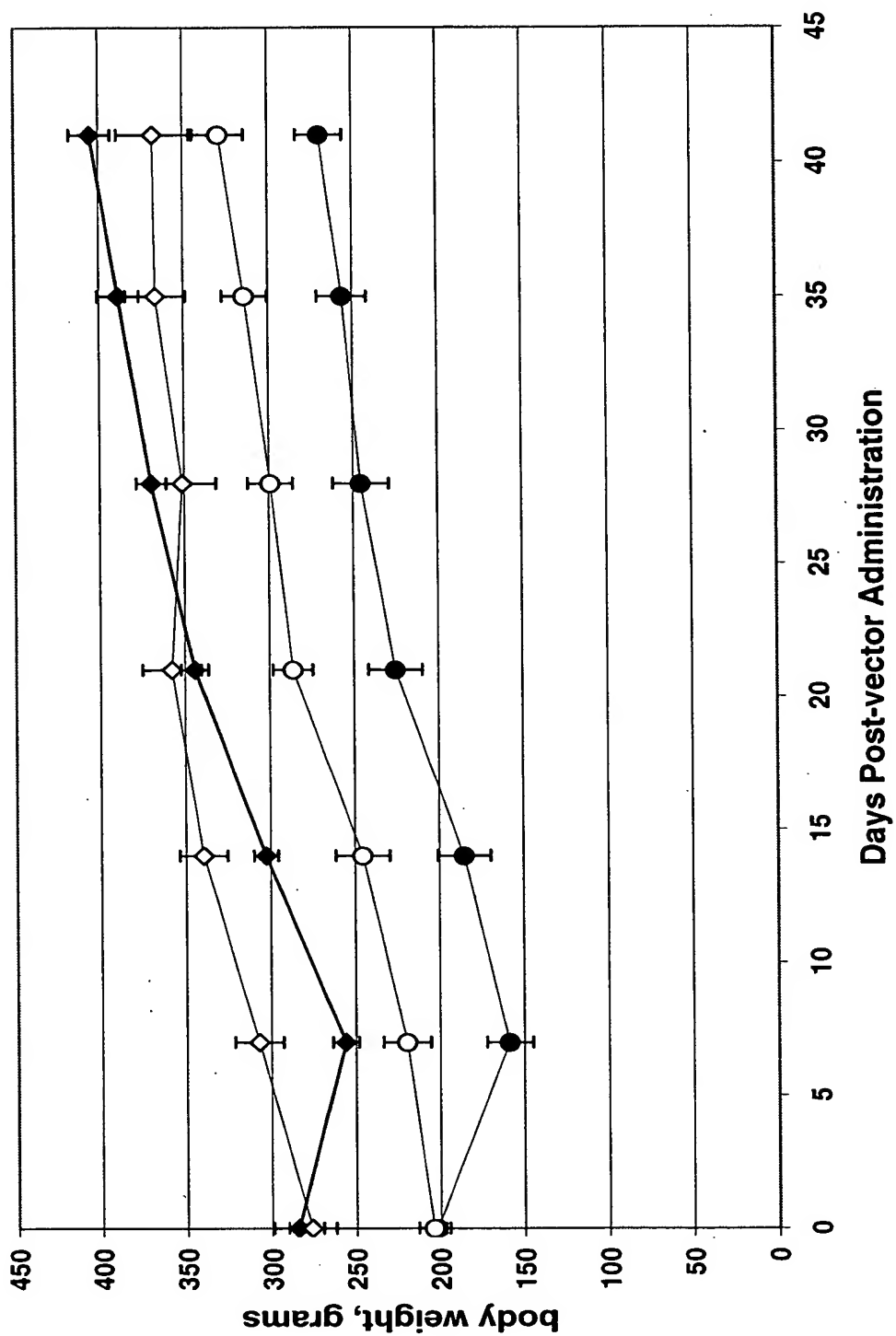


Figure 36

